Clinical Investigation

Abnormal Cellular and Humoral Immunity in Childhood Acute Lymphoblastic Leukemia in Long-term Remission

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The effects of multiagent chemotherapy were evaluated in 36 patients with acute lymphoblastic leukemia in long-term remission. Decreased numbers of neutrophils, lymphocytes, T cells and T-cell subpopulations (T_M, T_G, T4 [helper] and T8 [suppressor]) were found. Additional abnormalities were reduced numbers of natural killer (NK) cells, assayed using the myeloid/erythroid K562 cell line, and impaired immunoglobulin production by pokeweek mitogen (PWM)-stimulated, cultured mononuclear cells. Patients tested a year after chemotherapy was stopped showed a return to normal levels of neutrophils, lymphocytes, T, T_M and T_G cells. NK cells and the T4 cells, however, remain low, whereas the T8 cell numbers return to normal, and the helper to suppressor cell ratio remains abnormal for about three years. In experiments on PWM-stimulated cocultured cells, immunoglobulin production was decreased and abnormal T-cells, defective suppressor cell immunoregulation and relatively normal B-cell function were detected. The abnormal findings were probably the result of the multiagent chemotherapy.

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The improved long-term survival and the possible cure of children with acute lymphoblastic leukemia (ALL) raise the question of the occurrence of long-term abnormalities in the immune system caused by intense and prolonged multiagent chemotherapy. Immunosuppression may predispose a patient to infection,[1-3] some fatal,[2] or secondary complicating malignant disorders.[4,5] Investigations of the immune system after cessation of chemotherapy have shown a decreased number of circulating leukocytes[6-8] and a rebound increase in lymphoid cells to near-normal levels three to six months later.[8] Leventhal and co-workers have reported that the chemotherapy in childhood ALL caused a mild T-cell dysfunction and a more profound depression of B cells, with some recovery after a year.[9] Others have shown that the documented decrease in immunoglobulins and antibody production increased to near-normal levels after the first year off treatment.[6,7] It does, therefore, seem that in the year following the cessation of multiagent chemotherapy, both cellular and humoral immunosuppression recover to near normal.[10] To the contrary, Layward and associates have reported prolonged T- and B-cell abnormalities even five years after stopping treatment.[11]

To further examine the long-term effects of multiagent chemotherapy in children with ALL, we evaluated patients while they were receiving multiagent chemotherapy and after stopping treatment. The numbers of leukocytes, neutrophils, lymphocytes and T cells and T-cell subpopulations T_M and T_G cells are decreased while a child is on chemotherapy and return to normal after the first year off treatment. Monoclonal antibody levels (T4 and T8) tested in treated patients were abnormal and in four patients who were retested, they returned to normal only after three years. Multiagent chemotherapy substantially depressed natural killer (NK) cell activity and pokeweed mitogen (PWM)-stimulated immunoglobulin synthesis. NK cell activity rebounded after treatment was stopped, but after six months it decreased to lower than normal levels. PWM-stimulated immunoglobulin synthesis was abnormal during the first year off treatment, and the defect appeared to be caused by a decreased number of helper cells. Allogeneic cocultures showed defective T cells and an unusual sensitivity of B cells to allogeneic normal T-cell immunosuppression.

Patients

A total of 36 patients with ALL who were younger than 14 years at diagnosis were studied: 11 were on chemotherapy and 25 were off treatment. The patients receiving chemotherapy were all in first remission for one to two years and the patients off treatment included 20 in first remission, 4 in second remission and 1 in third remission. The ages of the patients on chemotherapy were 3 to 12 years and off treatment 11 to 22 years. Evaluations were carried out in 12 patients

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ABBREVIATIONS USED IN TEXT
ALL = acute lymphoblastic leukemia
FITC = fluorescein isothiocyanate
Ig = immunoglobulin
NK = natural killer (cells)
PWM = pokeweed mitogen

within the first year (mean 10 months, range 8 to 12 months) and in 13 after the first year off treatment (mean 17 months, range 15 to 21 months). PWM-stimulated immunoglobulin production was tested in five patients receiving chemotherapy, in eight less than one year off treatment and in ten more than one year off treatment, and in six of these patients the test was repeated both before and after one year. During the study period of four years, none of the patients have relapsed; in one, however, a secondary complicating acute nonlymphocytic leukemia has developed. At the time of treatment of 30 of the patients, no set protocol was followed and the patients were treated with multiagent chemotherapy for at least five years; all received standard induction therapy with vincristine sulfate and prednisone, L-asparaginase was added in four and all received the standard maintenance regimen of 6-mercaptopurine, once-a-week doses of methotrexate and monthly pulses of vincristine and prednisone.

The study included an additional six patients who had been placed on Children's Cancer Study Group protocols (the Children's Cancer Group 160 series) and received similar multiagent chemotherapy. These six patients received treatment, one for 4 years (including one relapse), one for 3½ years and four for 3 years. The laboratory tests were carried out after stopping treatment and the results were compared with those of the larger group of patients. Seven of the patients off treatment (including five patients who had relapsed) also received cyclophosphamide, and in two of the five patients who relapsed, doxorubicin (Adriamycin) hydrochloride was added to their regimen. All patients received central nervous system prophylaxis consisting of intrathecal administration of methotrexate and cranial irradiation with 2,400 rads, the patients on the Children's Cancer Study Group protocols receiving only 1,800 rads. None of the patients studied showed poor prognostic criteria at their initial presentation.

Control blood specimens were collected from adult laboratory staff aged 18 to 40 years. Informed consent was obtained from all participants and their parents in accordance with the Human Subjects Review Board at the University of California, Irvine, and Childrens Hospital of Orange County (Orange), California.

Methods

For all patients, complete blood counts were done on a Coulter counter and the absolute numbers of cells were calculated from the differential counts. The blood specimens were drawn from patients on chemotherapy at the time of the monthly intravenous pulses of chemotherapy and from patients off treatment at the time of a routine clinical evaluation.

Separation and Enumeration of T and Non-T Cells

Peripheral blood (10 to 15 ml) collected in sterile heparinized tubes was diluted 1:1 with calcium and magnesium in a free Hanks balanced salt solution, and mononuclear cells were separated on a Ficoll-Hypaque density gradient (1.074) according to the method of Böyum.[12] T cells were enumerated as E rosettes by a previously described modification[13] of the method of Wybran and colleagues.[14] T and non-T cells were purified by a modification of the method of Gupta and Good.[15] Mononuclear cells at 2×10^6 cells per ml were incubated with 1% sheep erythrocytes at 4°C overnight. An aliquot was tested for the percent of sheep erythrocyte rosetting (T cells), and the remaining cells were layered onto a Ficoll-Hypaque cushion and centrifuged at $400 \times g$ for 25 minutes. The pellet contained rosetted T cells and at the interface the non-T cells. The T-cell population was treated with water to lyse the sheep erythrocytes, and both cell populations were isolated and then washed three times with Hanks balanced salt solution. The percent of B cells was estimated by a modification of the direct fluorescent antibody technique[16] previously described[13] but using fluorescein isothiocyanate (FITC)-goat anti-human immunoglobulin $F(ab)'_2$ (Kallested, Chaska, Minn). The fluorescent B cells were enumerated under an Olympus fluorescent microscope, with at least 200 cells being counted.

T-Helper (T_M) and T-Suppressor (T_G) Cells

The percent of T_M and T_G cells was determined by incubating T cells overnight at 37°C in RPMI-1640 medium (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (Reheis, Phoenix), 2 mmol per liter glutamine, 25 mmol per liter HEPES buffer, 100 units penicillin and 100 units streptomycin per ml (all from Gibco).[15] Cells were suspended in phosphate-buffered saline solution at 7×10^6 cells per ml, and 0.1 ml of T cells was added to 0.1 ml of 2% washed ox erythrocytes coated with immunoglobulin (Ig) M or IgG from rabbit anti-ox erythrocyte serum (Cappel, Cochranville, Penn). The cell mixture was incubated at 4°C for 90 minutes. Rosetted cells were counted under the microscope, with at least 200 cells being evaluated. The absolute number of T_M and T_G cells was calculated from the absolute T-cell number.

T-Helper and T-Suppressor Cells (Monoclonal Antibodies T4 and T8) and Helper:Suppressor Cell Ratio

Ficoll-Hypaque-separated mononuclear cells were resuspended at 1×10^6 cells per ml in RPMI-1640 medium containing 2% fetal calf serum. One milliliter of cells was pelleted in a test tube at $500 \times g$ at 4°C for six minutes. The supernatant was removed and 200 μl of diluted monoclonal antibody (Coulter, Hialeah, Fla) was added to the cell pellet and vortexed gently. After 30 minutes of incubation on ice, cells were washed twice with RPMI-1640 and 2% fetal calf serum, and 200 μl of diluted goat anti-mouse immunoglobulin antibody conjugated with FITC (Kallested) was added to the cell pellet. The cells were incubated 30 minutes on ice, washed three times, resuspended in a minimal volume of RPMI-1640 and mounted on microscope slides. The number of fluorescent cells of 200 lymphocytes counted under a fluorescent Olympus microscope was recorded as a percentage.

Natural Killer Cell Assay

The target cell for the natural killer cell assay[17] was the K562 cultured cell line (obtained from Sloan-Kettering Institute, New York) grown in RPMI-1640 supplemented with 10% fetal calf serum. A total of 2×10^6 cells was washed once and incubated in 0.2 ml with 0.2 mCi sodium chromate Cr 51 (ICN, Irvine, Calif) for one to three hours at 37°C. After incubation, the cells were washed three times in 2% fetal calf serum RPMI-1640. Effector cells were mononuclear cells obtained from blood specimens of patients and normal sub-

jects. The effector cells were incubated with 1×10^4 target cells for three hours at 37°C at 100:1, 50:1, 40:1, 20:1 and 10:1 effector-to-target cell ratios. Controls for spontaneous ^{51}Cr release and total ^{51}Cr release by the addition of 5% tyloxapol (Triton X-100) were included in the assay. After incubation ^{51}Cr release was determined as counts per minute in a γ-well-counter on 0.1 ml of supernatant. The percent of lysis was calculated by dividing normal counts per minute minus spontaneous counts per minute by total counts per minute minus spontaneous counts per minute. The mean of triplicate specimens was recorded. The percent of lysis was graphed at different effector-to-target cell ratios, and lytic units (one lytic unit is that number of cells required to cause 15% specific lysis) were calculated.

Pokeweed Mitogen-Stimulated Immunoglobulin Production

The result of T-cell and non-T-cell interaction was assessed by isolating T and non-T cells as previously described and estimating immunoglobulin production in PWM-stimulated cultures.[18] Autologous non-T cells at 5×10^5 cells per ml were mixed and cocultured with T cells at the T cell-to-non-T cell ratios of 2:1, 3:1, 4:1, 6:1, 8:1 and 10:1. The cells, suspended in supplemented RPMI-1640, were incubated in Falcon flat-bottom microtiter plates (Becton Dickinson, Oxnard, Calif) in 5% carbon dioxide with or without PWM for seven days at 37°C.

Allogeneic experiments were also done wherein normal T cells were substituted for patient T cells in the same ratios. Control data were obtained by comparing similar mixed cocultures of specimens taken from normal subjects. A modified solid-phase radioimmunoassay of Catt and Tregear was carried out on the supernatants to determine IgG and IgM concentrations.[19] Polyvinyl chloride microtiter "U" plates (Dynatech, Alexandria, Va) coated with goat anti-human IgG or IgM (0.5 mg per ml; Meloy, Springfield, Va) were incubated overnight at 4°C. Plates were then washed three times with 0.05% Tween-phosphate-buffered saline washing solution. A standard curve was generated using six concentrations of human immunoglobulin (Capell) from 0.5 ng per well to 10 ng per well. Appropriate dilutions of supernatants were added in triplicate and incubated overnight at 4°C. Plates were again washed three times in washing solutions, and goat anti-human IgG or IgM (Tago, Burlingame, Calif) labeled with iodine 125 (ICN) by the chloramine-T method[20] was added and the solution incubated overnight at 4°C. Plates washed three times in washing solution and eight times in water were dried, and individual wells were counted in a Searle γ-well-counter for 60 seconds. The radioactivity counts were calculated from a standard curve and converted to immunoglobulin production in nanograms per 2×10^6 non-T cells. The amount of immunoglobulin produced (IgG and IgM) was recorded as immunoglobulin in stimulated cultures minus immunoglobulin in unstimulated cultures.

Statistical analysis of the data was accomplished by the Student's *t* test, with *P* less than .05 considered significant.

Results

Cell Counts

Significantly reduced leukocyte, polymorphonuclear neutrophil and lymphocyte counts were found only in those patients on chemotherapy. A significantly reduced number of T cells was found in patients on chemotherapy and also in those less than one year off treatment. The number of T_M and T_G cells was significantly decreased in patients receiving chemotherapy and those less than one year off treatment. B-cell numbers were normal in all patient groups (Figure 1).

Helper and Suppressor Cells (Monoclonal Antibodies T4 and T8)

The results of nine patients on chemotherapy were analyzed in Table 1. The absolute numbers of lymphocytes are recorded in addition to percentages, as patients on chemotherapy have leukopenia and lymphopenia.[2] The patients receiving chemotherapy showed significantly reduced absolute numbers of both helper and suppressor cells (*P* .003), but the percentage of helper cells was normal whereas the percentage of suppressor cells was significantly increased ($P < .02$). The mean helper-to-suppressor cell ratio was within the normal range, with ratios less than 1.0 in only two patients.

Table 2 shows the data in the ten patients who were ten months to three years off treatment. Six patients tested were more than two years off treatment. A statistically significant decreased absolute number and percentage of helper cells (T4) were found, but the number of suppressor cells (T8) was normal. The percentage of helper cells was less than 30 in eight of the ten patients and in only one of the normal subjects ($P < .001$). The ratio of helper to suppressor cells was significantly reduced ($P < .001$) and less than 1.0 in five of the ten patients, whereas in all normal subjects the ratio was greater than 1.0. More recent data on four patients more than three years off treatment showed a normal ratio of helper to suppressor cells (Table 3).

Natural Killer Cells

The number of NK cells was significantly decreased in patients on chemotherapy. During the first six months off treatment, the NK cell number showed varying levels. Three of the six patients showed a rebound to greater than normal lytic units and three had low levels. After six months, a statistically significant decrease in circulating NK lytic units per milliliter was found in the 14 patients tested (Table 4).

Pokeweed Mitogen-Stimulated Immunoglobulin Production

In autologous experiments, initially 2:1, 3:1 and 4:1 ratios were compared. IgG production was significantly reduced in the coculture ratios 2:1, 3:1 and 4:1 (T and non-T cells) in patients on chemotherapy when compared with that of controls. In patients less than one year off treatment, IgG production was significantly decreased in the coculture ratios 2:1 and 3:1, but at a 4:1 ratio IgG production increased to normal levels. In the patients off treatment for greater than a year, IgG production was significantly decreased only in the coculture ratio 2:1 (Figure 2). When compared with controls, IgM production (Figure 3) was significantly decreased in the coculture ratios 2:1, 3:1 and 4:1 in both patients on chemotherapy and those less than a year off treatment. In patients off treatment for more than a year, IgM production was significantly decreased only in the coculture ratio 2:1. Sequential comparison of PWM-stimulated immunoglobulin production in six patients off treatment for less and more than a year is shown in Table 5. After a year off treatment, IgG production increased in four patients: patient 3 showed a rise only at the 4:1 ratio, patient 5 did not show a rise in the IgG production after 15 months off treatment and patient 6 showed only a slight increase in IgG production after 18 months off treatment. Increased IgM levels were

found in all the patients tested after a year off treatment. Analysis of patients who also received cyclophosphamide is detailed in Table 6. No significant differences in PWM-stimulated IgG and IgM levels were found when compared with patients who did not receive cyclophosphamide. When significant differences were seen in the initial ratios 2:1, 3:1 and 4:1, eight patients from ten months to four years off therapy were studied at the higher T-cell to non-T-cell ratios of 6:1, 8:1 and 10:1 to determine when suppression occurred. In autologous experiments, IgG production by PWM-stimulated mononuclear cells showed an exponential rise when T-cell to non-T-cell ratios were increased from the 2:1 to 6:1 ratios (Figure 4). Suppression of the IgG production was noted when the ratios were 8:1 and 10:1. The peak levels occurred at the 6:1 ratio in the patients, whereas the IgG levels peaked at 3:1 or 4:1 ratios in the normal subjects. Suppression was noted at the 8:1 ratio in the patients and 6:1 in the normal subjects.

IgM production (Figure 5) was greatly decreased in the patient group compared with the normal control subjects. In

TABLE 1.—*Decreased Helper Cells With Normal Helper (H) and Suppressor (S) Cell Ratios in 9 Patients on Chemotherapy*

	Helper Cells (%T4)				Suppressor Cells (%T8)				H:S Ratio	
	Controls		Patients		Controls		Patients		Controls	Patients
	%	Abs No.	%	Abs No.	%	Abs No.	%	Abs No.		
	39	570	55	186	31	741	31	105	1.2	1.8
	45	1,215	35	277	21	567	17	135	2.1	2.1
	39	1,510	46	397	18	342	18	156	2.0	2.6
	29	406	35	314	21	294	42	376	1.4	0.8
	38	950	26	274	20	500	27	285	1.9	1.0
	35	665	36	140	12	228	20	18	2.9	1.8
	41	861	39	213	18	378	41	224	2.3	1.0
	34	646	19	109	19	361	25	303	1.8	0.4
	31	589	32	352	11	209	14	154	2.8	2.3
	25	475			22	418			1.1	
	31	589			23	437			1.4	
Mean	35	771	39	257	20	407	28	194	1.9	1.6
SD	6	337	9	86	5	154	11	107	0.6	0.7
*P**			.157	.001			.02	.003		.15

Abs No.=absolute numbers, SD=standard deviation

**P* values obtained by comparing percentages and absolute numbers for normal subjects versus patients ($P < .05$ considered significant).

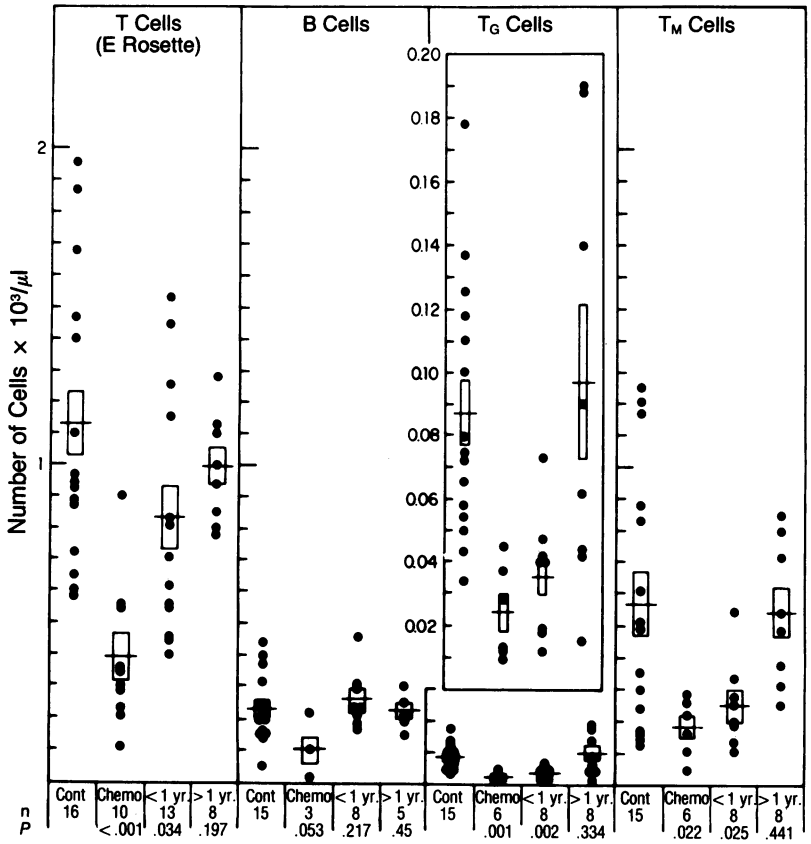

Figure 1.—Absolute number of T cells, B cells and the subpopulations of T_G and T_M cells in controls (Cont; normal subjects) and patients on chemotherapy (Chemo.) less than and greater than 1 year off treatment. A 10 x magnification of the results of the T_G cells has been inserted. Differences between the mean and standard error of the mean of controls and patient groups were evaluated by the Student's *t* test (*P* values of $< .05$ are considered significant).

four patients, IgM production was not suppressed even at the 10:1 ratio, and a steady rise of IgM was recorded. One patient who was 2½ years off treatment showed a plateauing of IgM levels at the 6:1 and 8:1 ratios (946 ng per 2×10^6 cells) and a

TABLE 2.—*Depressed Helper Cells and Abnormal Helper (H) to Suppressor (S) Cell Ratios in 10 Patients Off Treatment*

	Helper Cells (%T4)				Suppressor Cells (%T8)				H:S Ratio	
	Controls		Patients		Controls		Patients		Controls	Patients
	T4,%	Abs No.	T4,%	Abs No.	T8,%	Abs No.	T8,%	Abs No.		
	39	570	15	285	31	741	19	361	1.2	0.8
	45	1,215	27*	459	21	567	26	442	2.1	1.0
	39	1,510	19	361	18	342	15	285	2.0	0.8
	29	406	36	864	21	294	23	552	1.4	1.6
	38	950	18*	342	20	500	26	494	1.9	0.7
	35	665	8*	160	12	228	13	260	2.9	0.6
	41	861	19†	342	18	378	21	378	2.3	0.9
	34	646	27†	540	19	361	20	400	1.8	1.4
	31	475	26*	520	11	209	33	660	2.8	0.8
	25	589	30	510	22	418	27	459	1.1	1.1
	31	475			23	437			1.4	
Mean	35	771	23	438	20	407	22	429	2.0	1.0
SD	6	337	8	191	5	154	6	121	0.6	0.3
P Value			.003	.0066			.15	.36		.0003

Abs No.=absolute number of cells times 10^3 per μl, SD=standard deviation

*Patients off treatment for more than 2 years.
†Patients off treatment for more than 3 years.

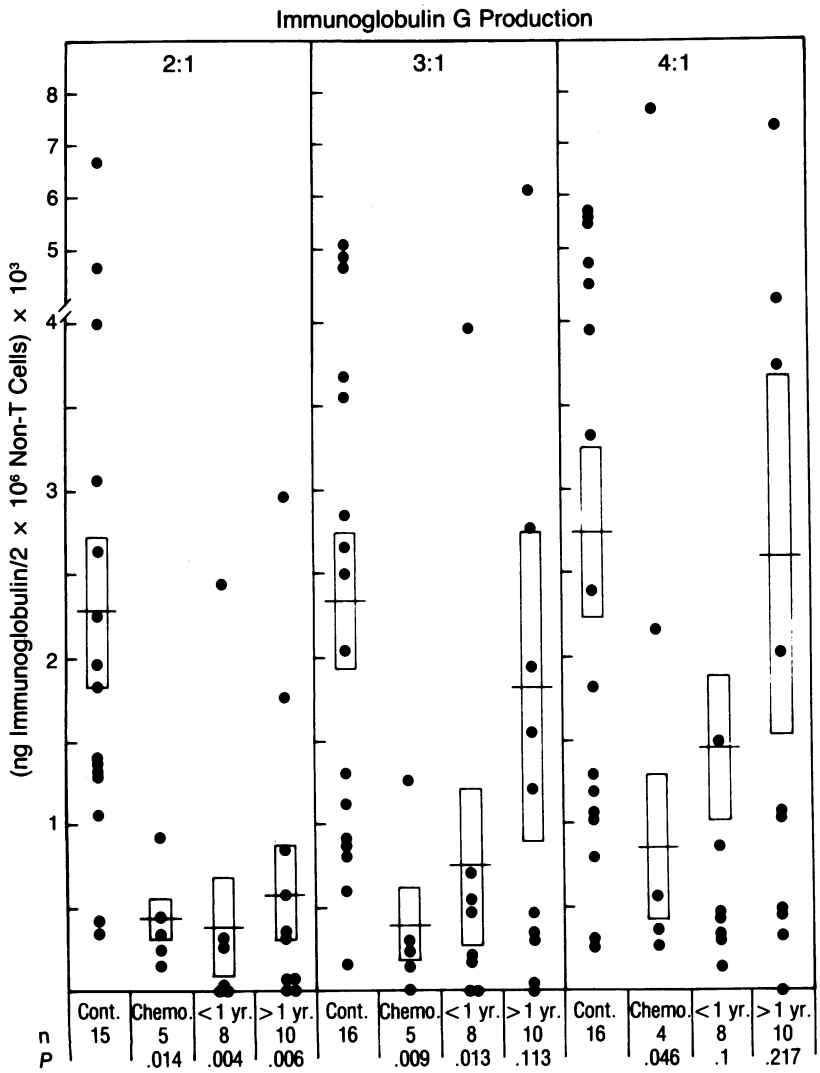

Figure 2.—The graph shows the amount of immunoglobulin (Ig) G produced in pokeweed mitogen-stimulated mononuclear cell cocultures with increasing T cell to non-T cell ratios. Differences between the mean and the standard error of the mean of the controls (Cont; normal subjects) and the patient groups on chemotherapy (Chemo.) and off treatment for less than and greater than 1 year were evaluated by the Student's *t* test (*P* values < .05 are considered significant).

slight decrease at the 10:1 ratio (547 ng per 2×10^6 cells). In three patients (3, 3½ and 4 years off treatment), plateauing of IgM occurred at the 4:1 and 6:1 ratios (mean 110 ng per 2×10^6 cells) and IgM production was suppressed at the 8:1 ratio (mean 710 ng per 2×10^6 cells). In the normal controls, IgM production was similar to IgG production and suppression was noted at the 6:1 ratio.

In allogeneic experiments, incubating normal T cells with patients' non-T cells evoked a substantial decrease of immunoglobulin production. Both IgG and IgM were affected, with suppression occurring at the 3:1 ratio, and greatly decreased at the higher ratios of 6:1, 8:1 and 10:1 (Figures 4 and 5). Control data were obtained from six experiments wherein allogeneic normal cells were cocultured in the same way. The results showed that IgG and IgM production paralleled the normal autologous cocultures.

Discussion

The highly successful treatment of childhood acute lymphoblastic leukemia has resulted in an immune deficiency, documented by many workers.[6-9,11] It is possible that this immune deficit is caused solely by multiagent chemotherapy. In this study we investigated the effects of multiagent chemotherapy on patients with leukemia and found greatly decreased total leukocyte, neutrophil and lymphocyte counts. T cells and T-cell subpopulations—T_M, T_G, T4 and T8—were also decreased. After stopping treatment, improvement to near-normal levels occurred after the first year off treatment. While receiving chemotherapy, patients have reduced numbers of helper cells (T4) and suppressor cells (T8) and normal helper-to-suppressor cell ratios, indicating that helper and suppressor cells are equally affected by chemotherapy. After stopping treatment, prolonged significantly decreased levels of T4 cells, normal T8 cell levels and abnormal helper:suppressor cell ratios were found. This indicates that the T-suppressor cells are the first to rebound and the T helpers return to normal levels at a slower rate. The abnormalities resolved in four patients three years after treatment was stopped.

Further studies showed that NK cells were decreased while a patient was receiving chemotherapy and after treatment was stopped, with the number of NK lytic units either increased or decreased during the first six months off treatment, then falling to lower than normal levels. McGeorge and co-workers previously reported that multiagent chemotherapy affected in vitro NK cell activity, but long-term effects were not found.[21] The decreased number of NK lytic

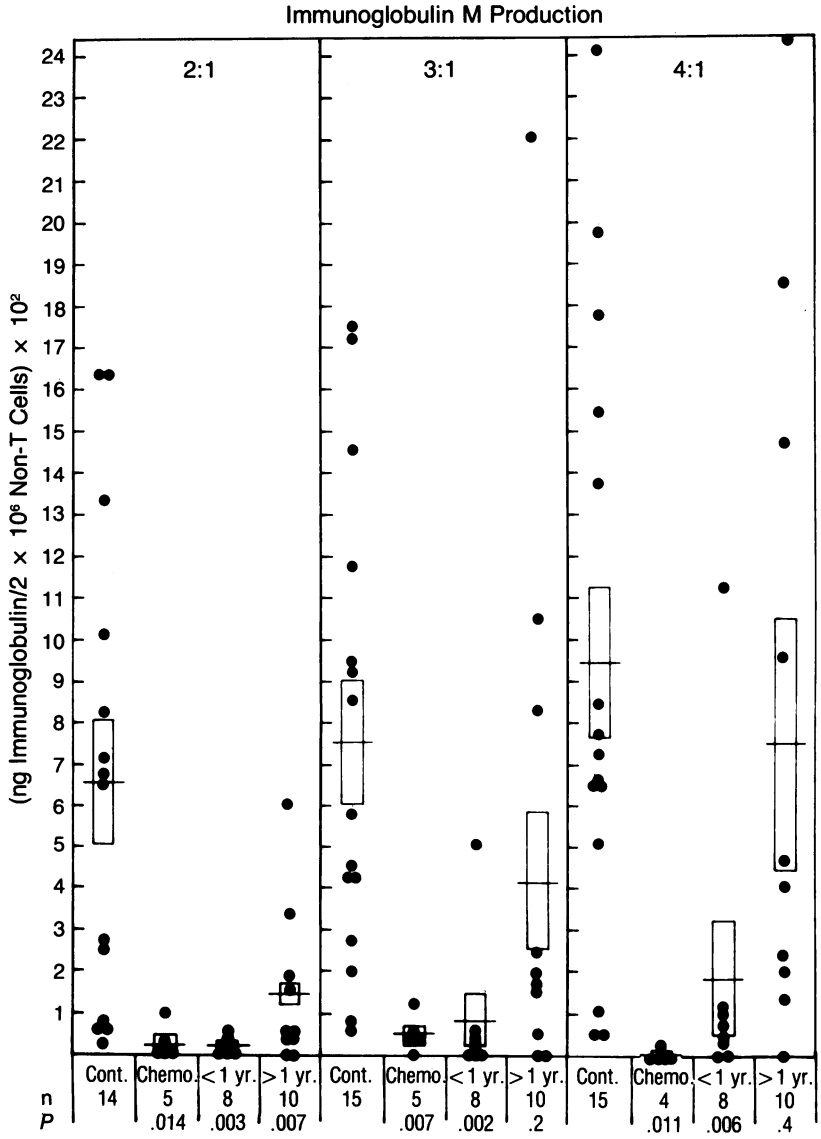

Figure 3.—The graph shows the amount of immunoglobulin (Ig) M produced in pokeweed mitogen-stimulated mononuclear cell cocultures with increasing T cell to non-T cell ratios. Differences between the mean and the standard error of the mean of the controls (Cont; normal subjects) and the patient groups on chemotherapy (Chemo.) and off treatment less than and greater than 1 year were evaluated by the Student's *t* test (*P* values < .05 are considered significant).

units detected in our study, however, may have some clinical relevance and confirms the decreased NK cell activity previously reported by Matera and Giancotti.[22]

In the normal subjects, IgM production peaked at the 4:1 ratio, whereas in four of the eight patients IgM production was grossly abnormal and normal IgM levels were detected only when 8:1 and 10:1 ratios were attained. Suppressor cell function and suppression were near normal for IgG regulation, with decreased levels at an 8:1 ratio in patients compared with a 6:1 ratio in the normal subjects. Grossly abnormal suppressor cell suppression of IgM production was found, however, and in four of eight patients IgM production was not abrogated even at 8:1 and 10:1 ratios. Decreased IgM production has been previously reported in patients with Hodgkin's disease receiving multiagent chemotherapy.[23]

In addition, our studies showed abnormal B-cell function. In cocultures of patients' B cells with allogeneic T cells, pronounced immunosuppression of both IgG and IgM production was found. This suggests that patients' B cells are abnormally sensitive to normal suppressor cell regulation. The significance of these immunologic abnormalities is not known. It is known, however, that immune disorders are associated with malignant conditions, and it can be questioned whether these patients will be prone to malignancy in later years. It has been reported that the suppressor cell system is implicated in various disease processes ranging from excessive suppressor cell activity in chronic lymphatic leukemia,[24] hypogammaglobulinemia[18] and selected IgA deficiency,[25] a suppressor cell dysfunction in systemic lupus erythematosus[26,27] and deceased activity in autoimmune disorders.[28-30] Suppressor T cells have also been shown to play a role in the immunologic enhancement of tumor growth and immune elimination of tumor cells by the host.[31] Suppressor cell dysfunction with altered immunoregulation was reported in a patient with microglobulinemia and his kindred.[32] Aberrant suppressor T cells have been reported in patients with graft-versus-host disease following bone marrow transplanta-

TABLE 3.—*Number of Helper (T4) and Suppressor (T8) Cells and T4:T8 Ratios in 4 Patients Reevaluated After 3 Years Off Treatment**

	Off Treatment 1-2 Years			After 3 Years		
	T4 Number	T8 Number	T4:T8 Ratio	T4 Number	T8 Number	T4:T8 Ratio
	27	26	1.0	45	25	1.8
	18	26	0.7	35	33	1.1
	19	21	0.9	37	21	1.8
	30	24	1.2	36	17	2.1
Mean	24	25	0.9	38	24	1.7
SD	6	3	0.2	5	7	0.4
P Value	.001	.1	.001	.1	.1	.1

SD=standard deviation

*Normal control data—see Table 1.

TABLE 4.—*Natural Killer Cell Activity in 7 Patients on Chemotherapy and 14 Off Treatment**

	Controls	On Chemo	<6 Months Off Chemo	>6 Months Off Chemo
	6.7	0.5	33.3	5.8
	17.8	1.1	45.0†	16.6†
	9.3	2.4	14.3†	8.5†
	18.0	2.0	6.4†	7.8†
	23.3	0.7	4.2	4.5
	37.1	1.0	2.0	8.5
	11.4	2.5		10.4
	5.3			9.6
Mean	16.2	1.5	17.5	9.0
SD	10.1	0.8	17.6	3.6
n	8	7	6	8
P Value		.001	.4262	.045

Chemo=chemotherapy, SD=standard deviation

*In circulating lytic units per milliliter blood. 1 lytic unit=15% lysis
†Patients reevaluated before and after 6 months.

TABLE 5.—*Sequential Comparison of Pokeweed Mitogen-Stimulated Immunoglobulin Production in 6 Patients Less Than 1 Year and Greater Than 1 Year Off Treatment*

Patients	Ratio of T Cells to Non-T Cells	Immunoglobulin (Ig) Production IgG <1 Year	IgG >1 Year	IgM <1 Year	IgM >1 Year
1	3/1	197	6,175	34	1,050
2	...	184	1,563	12	176
3	...	478	186	0	65
4	...	561	1,210	40	235
5*	...	704	308	64	200
6	...	0	480	0	10
1	4/1	825	7,375	124	1,866
2	...	353	2,048	53	144
3	...	456	715	0	322
4	...	350	1,068	112	475
5*	...	427	361	80	240
6	...	200	494	0	961

*Patient 5 showed no rise in IgG levels.

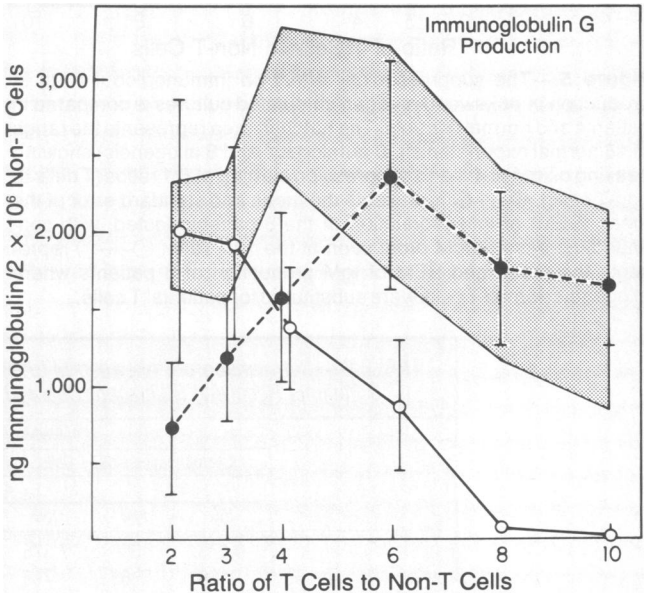

Figure 4.—The suppressor cell effect on immunoglobulin (Ig) G production in pokeweed mitogen-stimulated cultures is compared in patients and normal controls. The hatched area represents the range of 18 normal experiments (10 autologous and 8 allogeneic) showing peaking of IgG at 4:1 and suppression starting at 6:1 ratios (T cells to non-T cells). ●——● represents the mean and standard error of the mean (SEM) of IgG production of the 8 patients tested with peaking of IgG production at 6:1 and immunosuppression at 8:1 and 10:1 ratios; ○——○ represents the mean and SEM of IgG production in 8 patients where allogeneic normal T cells were substituted for patients' T cells.

tion,[33,34] and this state is associated with a higher than normal incidence of malignant disorders. The impaired suppressor cell activity, mild for IgG suppression and more substantial for IgM immunosuppression, may be accounted for by postulating that a critical level of immunoglobulin production is required for suppressor cell function to be "turned on" or helper activity to be "switched off." It is also evident that suppressor cell activity is present, but there is a delay in the reactivation of IgM suppression. The preliminary findings require further confirmation and investigation. In the follow-up of these patients over the three-year period of the present study, none of these patients has relapsed. In one of the 19 patients, however, a secondary complicating leukemia recently developed, which may mean that when an immune-compromised state reverts to normal, a susceptibility to the development of secondary (complicating) malignant tumors may result. Further long-term follow-up is required to determine the significance of the abnormal chemotherapy-induced cellular and humoral immunity in a patient with childhood leukemia in long-term remission. To date, the patients as a group have done well. There have not been major infections developing after chemotherapy was stopped. This may have been expected given the increased percentage of suppressor cells, a decreased number of helper cells and a decreased number of natural killer cells. It appears that the impaired immune state did not significantly increase the severity or frequency of infections the year after chemotherapy was stopped.

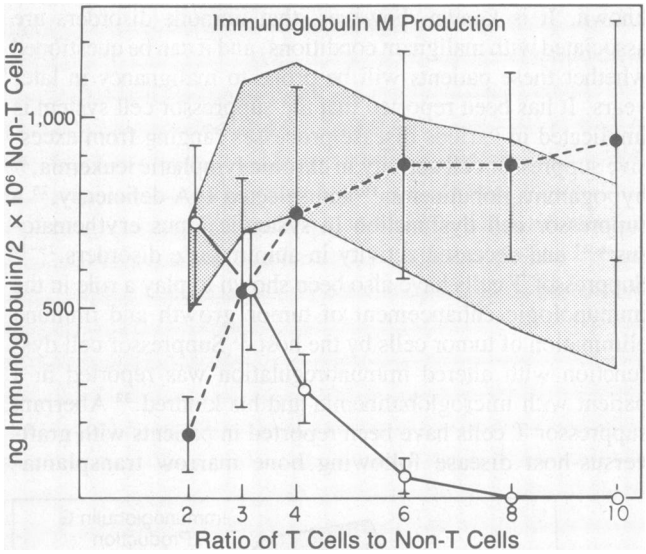

Figure 5.—The suppressor cell effect on immunoglobulin (Ig) M production in pokeweed mitogen-stimulated cultures is compared in patients and normal controls. The hatched area represents the range of 18 normal experiments (10 autologous and 8 allogeneic) showing peaking of IgM at 4:1 and suppression starting at 6:1 ratios (T cells to non-T cells). ●—● represents the mean and standard error of the mean (SEM) of IgM production of the 8 patients tested, with continued increase of IgM production at the 10:1 ratio; ○—○ represents the mean and SEM of IgM production of 6 patients where allogeneic normal T cells were substituted for patients' T cells.

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TABLE 6.—*Comparison of Pokeweed Mitogen (PWM)-Stimulated Immunoglobulin Production in 9 Patients Receiving Multiagent Chemotherapy With and Without Cyclophosphamide*†*

	PWM-Stimulated Immunoglobulin Production‡							
	Cyclophosphamide, T Cells: Non-T Cells				No Cyclophosphamide, T Cells: Non-T Cells			
	3:1		4:1		3:1		4:1	
	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
	6,175	1,056	7,375	1,866	1,961	837	3,749	1,473
	186	65	715	322	1,563	176	2,048	144
	2,787	2,280	4,147	2,813	320	160	512	208
	308	200	361	240	1,210	253	1,068	475
	480	0	494	961				
Mean	1,987	720	2,618	1,240	1,264	357	1,844	576
SEM	1,152	434	1,382	489	350	162	710	308

SEM=standard error of the mean

*Patients more than 1 year off treatment, 5 received cyclophosphamide.
†*P* values not significant (>.05).
‡The values represent absolute numbers, as described in text.

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Medical Practice Question

EDITOR'S NOTE: *From time to time medical practice questions from organizations with a legitimate interest in the information are referred to the Scientific Board by the Quality Care Review Commission of the California Medical Association. The opinions offered are based on training, experience and literature reviewed by specialists. These opinions are, however, informational only and should not be interpreted as directives, instructions or policy statements.*

Vertebral Artery Surgery

QUESTIONS:

Under what conditions and for which diagnoses or symptoms is vertebral artery surgery indicated?

If the surgery is allowable, what diagnostic testing is indicated prior to surgery?

OPINION:

In the opinion of the Scientific Advisory Panels on General Surgery and Neurosurgery, the indications for vertebral artery surgery are relatively infrequent in the treatment of cerebrovascular disease. However, where vertebrobasilar insufficiency is clearly related to neurological signs and symptoms and is caused by a demonstrable intraluminal or extraluminal process, vertebral artery surgery is appropriate. Such conditions may include vascular occlusive disease, when one artery is occluded and the other is stenotic; aneurysms, resulting from trauma, arteriosclerosis or mycosis; vascular malformations, and elective occlusion or embolization in the treatment of vascular lesions of the posterior fossa. The type of surgical repair depends upon local anatomical and pathological factors.

The symptoms of vertebrobasilar insufficiency include but are not limited to vertigo, speech defects, bilateral visual defects, ataxia, transient hemiparesis (unilateral, bilateral or alternating), syncope and drop attacks. Often transient deficits of other cranial nerves are also present. Usually more than one symptom is present, and vertigo or dizziness alone should not be considered evidence of vertebrobasilar insufficiency.

Before surgery, a patient should have a thorough neurological examination and angiography of extracranial and intracranial circulation. Complete arch aortography and four-vessel angiography in multiple planes with serial views remain the diagnostic procedures of choice. Magnetic resonance imaging and digital subtraction angiography may also be helpful in some cases.